The Effect of Ginger on the Growth of *Lactobacillus Acidophilus* and *Bifidobacterium Bifidum* in Probiotic Dairy Products

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Abstract

Probiotics are regarded as live microorganisms that provide health benefits when consumed. In the present study, the potentiality of producing foods containing probiotic bacteria with milk, yoghurt and ginger bases was evaluated. The goal was to determine the effects of different doses of ginger on the growth of two probiotic bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together) in milk and yoghurt. The products were then examined in terms of pH, acidity, microbe counting and persistence. In day 14, the production quantities of the products were evaluated by sensory method. The results of the questionnaires (administrated to 30 people) in statistical–descriptive test were analyzed using SPSS software. It was observed that the control sample had the best taste. The bioability of probiotic bacteria was measured by direct counting method. Product permanence was determined within 21 days. It was revealed that the increased concentration of ginger had a positive effect on the growth of the probiotic bacteria in probiotic milk and yoghurt. To evaluate the effect of ginger on the amount of the probiotic yoghurt protein, the control sample and the yoghurt sample containing 3% ginger were tested. As the results revealead, the amount of protein in the sample with 3% ginger was more than the control sample.

Keywords: *Bifidobacterium bifidum*, ginger, *Lactobacillus aciophilus*, milk, probiotic, yoghurt

Introduction

Probiotics are live microbial food supplements that benefit the health of consumer by maintaining or improving their intestinal microbial balance. Due to their perceived health benefits, probiotic bacteria have been increasingly included in yoghurts and fermented milks during the past two decades. Most commonly, they have been lactobacilli such as *lactobacillus acidophilus*, and bifido bacteria are often referred to as "bifidus" (AFRC, 1998; Daly and Davis, 2008; Heller, 2001; Kudo, 2004; Saarela et al., 2000). A major development in functional foods pertain foods that contain probiotics and those which enhance health promoting microbial flora in the intestine. A

growing body of scientific evidence supports the concept that the maintenance of healthy gut microflora may provide protection against gastrointestinal disorders including gastrointestinal infections, inflammatory bowel disease and even cancer (Kudo, 2004; Mitsuoka, 1990; Saarela et al., 2000; Salminen et al., 1998).

Recently, the design and production of plant - based probiotic products have received much attention chiefly due to their natural health benefits (protein, fiber, vitamin and salts) and also because of the Variety in their production. Therefore, it seems that the issue of producing probiotic foods with appropriate qualities will be a major research topic for prospective researchers. Though, in the past decades, the synthetic chemical drugs that make use of separation mechanisms have been much in demand, their corresponding side effects are being gradually observed so much so that their irregular and improper consumption has turned out to be a critical issue (Daly and Davis, 2008; Jahanara and Haeriza, 2001; Saarela et al., 2000).

On the contrary, the benefits of medicinal plants and their little or zero side effects have made them a proper substitute, highly appreciated by physicians and patients especially by Iranian s as Iran possesses a very rich source of such plants and herbs used in the long run by Iranians (Jahanara and Haeriza, 2001).

Ginger is a medical plant that has been widely used in Chinese, Ayurvedic and Tibb-e- Unani herbal medicines all over the world, since antiquity, for a wide array of unrelated ailments (Afzal et al., 2001).

Currently, there has been a renewed interest in ginger, and several scientific investigations aimed at isolation and identification of active constituents of ginger, scientific verification of its constituents, and verification of the basis of the use of ginger in some of several diseases and conditions (Ali et al., 2008).

This article thus aimed at evaluating the effect of ginger on the growth of Lactobacillus *acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yoghurt in order to develop a new probiotic product with reinforcemen of probiotic effects.

Materials and Methods

In the present study, the materials included dried ginger powder, low fat sterilized milk, and yoghurt from supermarket (1.5% fat), lyophilized *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together) (CHR Hansen Company, Denmark) and MRS Agar (Merk Company, Germany).

A.The effect of ginger on the production of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together) in milk in the first phase

In order to produce milk which contain the probiotic bacterium *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together), four containers each containing 1 liter of low–fat sterilized milk (1.5% fat) were considered as our four groups. 0.33 gram starter (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*(together)) was added directly to all the containers, followed by adding ginger powder 0 (the control sample), 1, 2 and 3% to all the containers, respectively and finally they were placed in an incubator at 38 ^{o}C. The acidity test was performed approximately every 2 hours until reaching 42 ^{o}Dornic.

The samples were then taken out of the incubator and transferred to a refrigerator and stored at 2 ^{o}C. The produced probiotic milk was evaluated once every 7 days by counting the microbes, using direct counting method.

B.The Effect of ginger on the production of probiotic *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together) in yoghurt in the second phase

To Produce *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together) in yoghurt, after providing four containers, 1 liter of low-fat sterilized probiotic milk (1.5% fat) from the control group in the first phase, 15 gram (1.5%) starter of low-fat yoghurt and 0.33 gram (33%) starter (*Lactobacillus acidophilus* and *Bifidobacterium bifidum* (together) were added to each container. Different doses of ginger (0, 1, 2 and 3%) were added respectively to the containers and mixed properly so that ginger was uniformly dissolved. Afterwards, all the containers were placed in the incubator at 38 ^{o}C. Approximately every 2 hours, the acidity and pH tests were done until acidity reached 70 ^{o}Dornic. Then the samples were taken out of the incubator and transferred to the refrigerator and stored at 2 ^{o}C. The produced probiotic ginger yoghurt was evaluated every 7 days by counting the microbes, using direct counting method. After 7 days, the yoghurt was evaluated for sensory properties, using questionnaires administrated to 30 people. The respondents were asked to rate the factors of scent, taste and thickness on a scale ranging from very good to weak. The results were analyzed using a statistical descriptive test by SPSS version 17 software. To evaluate the effectiveness of probiotic yoghurt, kjeldahl method was used.

Results

Table 1 shows the pH level in the ginger *L. acidophilus* and *B. bifidum* (together) in milk and yoghurt during incubation, and Table 2 demonstrates the acidity level in the ginger *L. acidophilus* and B. *bifidum* (together) in milk and yoghurt during the same time. Table 3 shows the PH degree in ginger milk and ginger yoghurt *L. acidophilus* and *B. bifidum* (together) during the same cooling period, and Table 4 shows the acidity degrees of ginger milk and yoghurt *L. acidophilus* and *B. bifidum* (together) during the storage time in the refrigerator. Table 5 shows the growth rate of microbes in the ginger *L. acidophilus* and *B. bifidum* in milk and yoghurt, and Table 6 demonstrates the sensory evaluation of ginger *L. acidophilus* and *B. bifidum* (together) and yoghurt.

Table 1. The pH level in the ginger *L. acidophilus* and *B. bifidum* (together) in milk and yoghurt during incubation

pH Level										
Ginger Milk %	**00:00(h)**	**02:00(h)**	**02:15(h)**	**Ginger Yoghurt %**	**00:00(h)**	**02:00(h)**	**04:00(h)**	**04:20(h)**	**05:00(h)**	**05:20(h)**
0	5.49	4.42	4.38	0	5.56	5.25	4.65	↓	3.81	3.66

1	5.44	4.31	---	1	5.43	5.19	3.57	---	---	---
2	5.31	4.18	---	2	5.33	5.17	3.27	---	---	---
3	5.24	4.58	---	3	5.28	5.14	3.66	---	---	---

Table 2. The acidity level in the ginger *L. acidophilus* and *B. bifidum* (together) in milk and yoghurt during incubation

Acidity Level in Dornic Degree										
Ginger Milk %	**00:00(h)**	**02:00(h)**	**02:15(h)**	**Ginger Yoghurt %**	**00:00(h)**	**02:00(h)**	**04:00(h)**	**04:20(h)**	**05:00(h)**	**05:20(h)**
0	20	40	42	0	20	25	40	↓	60	70
1	22	44	---	1	24	30	73	---	---	---
2	28	48	---	2	27	33	109	---	---	---
3	28	42	---	3	30	31	60	70	---	---

Table 3. The pH level in the ginger *L. acidophilus* and *B. bifidum* (together) in milk and yoghurt within 21-day storage in the refrigerator

pH Level							
Ginger Milk %	**7 day**	**14 day**	**21 day**	**Ginger Yoghurt %**	**7day**	**14day**	**21day**
0	5.55	5.4	5.47	0	5.6	5.14	5.23
1	5.54	5.50	5.37	1	4.49	5	5.03
2	5.35	5.33	5.29	2	4.87	4.92	4.88
3	5.63	5.41	5.41	3	4.95	5.02	4.95

Table 4. The acidity level based on Dornic degree in the ginger *L. aciolophilus* and *B. bifidum* (together) in milk and yoghurt within 21-day storage in the refrigerator

Acidity Level in Dornic Degree							
Ginger Milk %	**7 day**	**14 day**	**21 day**	**Ginger Yoghurt %**	**7day**	**14day**	**21day**

0	66	83	80	0	98	88	90
1	70	76	89	1	109	99	94
2	97	86	96	2	119	107	110
3	80	81	109	3	121	102	102

Table 5. Growth of microbes in the ginger *L. acidophilus* and *B. bifidum* (together) in milk and yoghurt

Ginger Milk %	**10^{-5} cfu/gr**	**Ginger Yoghurt %**	**10^{-5} cfu/gr**
0	4.375×10^{11}	0	7.5×10^{11}
1	5.3×10^{11}	1	18.75×10^{11}
2	8.75×10^{11}	2	5×10^{11}
3	13.75×10^{11}	3	15×10^{11}

Table 6. Sensory evaluation of ginger *L. acidophilus* and *B. bifidum* (together) and yoghurt

Ginger yoghurt (%)	**Taste**	**Aroma**	**Thickness**	**Color**
0	120	119	110	134
1	104	109	110	111
2	79	96	94	87
3	67	87	88	81

Discussion

Diet is regarded as a major focus of public health strategy aimed at maintaining optimum health throughout the life, preventing early onset of chronic diseases such as gastrointestinal disorders, cardiovascular disease, cancer, osteoporosis, as well as promoting healthier ageing. Though the highly complex relationship between food and health is still poorly understood, recent research advances in different disciplines provide promising new approaches to improve our understanding. The growing demand for "healthy" food is a stimulating innovation and a new product development in the food industry, internationally. The food industry has a central role in facilitating healthier eating practices through the provision and promotion of healthy foods.

In recent years, the probiotic bacteria, as the food additives, have been introduced into numerous foods, of which the dairy products especially yoghurt has played an important role in carrying these bacteria (such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*). Regularly eating of the sufficient amounts of the living

cells called “the minimum treatment” is required if the consumer is to benefit from the probiotic products. The daily recommended amount of the yoghurt containing 10^6 CFU.ml^{-1} probiotic bacteria is 100 gr. It is also very important to investigate the survival of these microorganisms within the interval between storage in the refrigerator and consumption (Heller, 2001; Lourens-Hattingh and Viljoen, 2001).

Essence medicinal plants and herbs play significant roles in the human life and have been very popular for long among the Iranians.The ginger, as a medicinal plant, has extensive effects of which the most notble include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, ementia, fever, infectious diseases and helminthiasis (Afzal et al., 2001; Ali et al., 2008; Jahanara and Haerizade, 2001).

Ginger is on the FDA's "generally recognized as safe" list. The characteristic fragrance and flavor of ginger result from volatile oils that compose 1-3% of the weight of fresh ginger, primarily consisting of zingerone, shogaols and gingerols with [6]-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) as the major pungent compound. Zingerone is produced from gingerols during drying, having lower pungency and a spicy-sweet aroma. Ginger has a sialagogue action, stimulating the production of saliva which makes swallowing easier. Ginger is a minor chemical irritant and, because of this, it was used as a horse suppository by pre-World War I mounted regiments for gingering.

In the present study, the effects of ginger on the growth of the bacteria *L. acidophilus* and *B. bifidum* in probiotic milk and yoghurt were investigated. The acidity, pH and survival of the bacteria in the ginger probiotic milk and yoghurt were evaluated at 2 h interval till reaching 42 ^{o}Dornic acidity degrees for milk and 70 ^{o}Dornic for yoghurt in the incubator at 38 ^{o}C and also within 21-day period of storage in the refrigerator.

The probiotic milk containing 2% ginger reached 42 ^{o}Dornic acidity much earlier than other samples, which was transferred to a refrigerator and stored at 2 ^{o}C. So, this sample had the most effect on the growth of bacteria during incubation.

The sample with 1% ginger and subsequently the sample with 3% and finally the control sample reached 42 ^{o}Dornic and transferred to the refrigerator. So, the control sample had a minimal effect on the growth of bacteria during incubation. The acidity of the milk product with plant (no bacteria) did not change. This sample was transferred to the refrigerator, too.

During the 21 days storage of milk samples in the refrigerator, the acidity level in the sample with 3% ginger was higher than others, and subsequently the samples with 2%, 1%, 0% (the control sample) and finally the milk product with plant (no bacteria) were higher, respectively. Thus during refrigeration, milk product with plant (no bacteria) and the control sample showed the most persistence. The milk sample containing 3% ginger had a minimal persistence.

In the direct couning method of bacteria, maximum number of microbes were observed in the milk smple with 1% ginger and subsequently the sample with 3% , milk product with plant (no bacteria), the sample with 2% and finally the control sample were more, respectively in the first week. Maximum colony growth was observed in

the samples with 2% ginger and subsequently the control sample, the sample with 1% and 3% ginger were more, respectively. The milk product with plant (no bacteria) had no colony growth. The samples containing probiotic yoghurt were transferred to the incubator at 38 ^{o}C to let the acidity reach 70 ^{o}Dornic. The probiotic yoghurt containing 2% ginger reached 70 ^{o}Dornic acidity earlier than others which was transferred to a refrigerator and stored at 2 ^{o}C. So, this sample had the most effect on the growth of bacteria during incubation. The sample with 3% ginger and subsequently the sample with 1% , yoghurt product with plant (no bacteria) and finally the control sample reached 70 ^{o}Dornic and transferred to the refrigerator. So, the control sample had a minimal effect on the growth of bacteria during incubation.

During the 21 days storage of yoghurt samples in the refrigerator, the acidity level in the sample with 2% ginger was higher than others, and subsequently the sample with 3%, yoghurt product with plant (no bacteria), the sample with 1% and finally 0% ginger were higher, respectively. Therefore during refrigeration, the control sample had the most duration and the yoghurt sample containg 2% ginger had a minimal duration.

In the direct counting method of the bacteria, maximum number of microbes were observed in the yoghurt sample with 1% ginger and subsequently the sample with 3%, 0% (th control sample), 2% and finally yoghurt product with plant (no bacteria) were more, respectively in the first week.

Maximum colony growth was observed in yoghurt product with plant (no bacteria) and subsequently the sample with 0% (the control sample), 3%, 2% and 1% were more, respectively.

Although the basic feature of the probiotic products consumption is their medicinal effects (biovalue), their associated sensory properties are also important. In other words, sensory properties rather than medicinal effects play the most important role in their daily consumption. Among the probiotic products, fermented ones, especially the probiotic yoghurt, is popular worldwide for its unique sensory properties (Heller, 2001; Lourens-Hattingh and Viljoen, 2001). In the present study, the sensory properties which were investigated in all the samples including the controls were aroma, scent, color, thickness and taste.

The results of the questionairs administrated to 30 people showed that the yoghurt samples with 0% (the control sample) and 1% ginger, yoghurt product with plant (no bacteria), the sample with 2% and 3% ginger were more popular in aroma, scent, color, thickness and taste, respectively.

To evaluate the effect of ginger on the amount of propiotic yoghurt protein, the control sample and the yoghurt sample containing 3% ginger were tested. It was observed that the mount of protein in the control sample and yoghurt sample containing 3% ginger were 2.90 and 3.21, respectively. So, the effect of ginger on the amount of probiotic yoghurt protein in the yoghurt sample contaning 3% ginger was more than testifier. The results of the studies addressing the probiotic bacteria have demonstrated the following:

The increased concentration of soya caused an increase in the microorganism growth and a rise in the acidity level which in turn resulted in shorter incubation time for the desired acidity (Marhamatizadeh et al., 2009).

The effect of honey on the growth of the above mentioned bacteria, introduced simultaneously into dairy products and drinks, was investigated and the results indicated that the yoghurt with only *Lactobacillus acidophilus*

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tasted more sourer than the yoghurt with both bacteria. The products containing *Bifidobacterium bifidum*, compared to those with *Lactobacillus acidophilus*, were with slower growth rate and also tasted less sour and were of longer permanence. They were not of favorable taste when honey concentration increased and the control was of the best taste among all the samples (Marhamatizadeh and Kazeroonian, 2009; Marhamatizadeh et al., 2010).

In another study addressing the effect of garlic on the bacterial growth, it was demonstrated that the increased garlic concentration promoted the growth of the bacteria in probiotic milk and yoghourt (Marhamatizadeh, 2012).

Conclusion

Some previous studies have been carried out on soy, honey, tarragon, cinnamon, spearmint, garlic, dill, chicory and etc. In the present study, comparing the results showed that ginger caused an increase in the growth rate of *bifidobacterium bifidum* and *lactobacillus acidophilus* in dairy products too.

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